

# Identification of a Hepatitis B Virus Genome in Wild Chimpanzees (*Pan troglodytes schweinfurthi*) from East Africa Indicates a Wide Geographical Dispersion among Equatorial African Primates†

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**DNAs from four wild chimpanzees (*Pan troglodytes schweinfurthi*) from eastern Africa were screened for 14 DNA viruses and retroviruses. Between two and three viruses were found in each animal. An entire hepatitis B virus (HBV) genome was amplified and sequenced from samples taken from one animal. This indicates that HBV is distributed across the entire range of chimpanzee habitats.**

The transfer of microbes between hosts has been occurring from time immemorial. Given their higher mutation rates, microbes are able to adapt rather readily to their hosts. Our closest relative, the chimpanzee, is host to a number of viruses homologous to human pathogens, with simian immunodeficiency virus of chimpanzees (SIVcpz)—which is isogenic with human immunodeficiency virus type 1—being a case in point (6–8, 10, 11, 20, 22). Here, samples from four dead wild chimpanzees in East Africa were screened for a number of DNA viruses and retroviruses.

Small tissue samples (~1 cm<sup>3</sup>) were taken in the field from the kidney (animal AK), liver (animal FG), or lung (animals RAS and SAD) and stored in 70% alcohol. Samples were progressively rehydrated in distilled water. DNA was extracted in a laboratory that had never handled any of the viruses listed in Table 1. Total DNA was resuspended in 10 mM Tris-HCl (pH 8) and 1 mM EDTA. Its quality was checked by amplifying the mitochondrial DNA control region (D loop) with a single primer pair. All samples yielded good PCR products. Sequencing showed that all four animals were unambiguously *Pan troglodytes schweinfurthi* (data not shown), which are found in central and eastern Africa between the Ubangui River and the Great Lakes.

With respect to the retroviruses, all four samples proved negative for SIVcpz after extensive analysis with five primer pairs. RAS was positive for simian T-cell leukemia virus type 1 (STLV-1), while FG, RAS, and SAD harbored simian foamy viruses (Table 1). All four animals were infected by the TT circinovirus (TTV), while the liver sample from one animal (FG) was positive by tests with primers specific for hepatitis B virus (HBV). All samples were negative for adeno-associated virus, simian virus 40, JC virus, BK virus, B19 parvovirus, poxviruses, and papillomaviruses (Table 1). When it was tested

with degenerate primers specific for the 3' end of herpesviral DNA polymerase (open reading frame 9), the lung sample from SAD proved to be positive for a virus related to the human Kaposi's sarcoma-associated gammaherpesvirus or human herpesvirus 8, which is not without precedent (5). Overall, between two and three viruses were isolated from each animal. As no sera were available, it was not possible to test for antibodies to any of these viruses or to other viruses.

HBV-like viruses are known to infect chimpanzees in West Africa (*Pan troglodytes verus*) and West Central Africa (*Pan troglodytes troglodytes* and *Pan troglodytes vellerosus*) (6, 9, 10, 14, 19, 21) and have been known to infect at least one western lowland gorilla (*Gorilla gorilla gorilla*) from Cameroon (6). Hence, the virus obtained from FG is apparently the first to be identified from an East African chimpanzee (*P. t. schweinfurthi*). A complete HBV genome was amplified in six fragments and sequenced. Phylogenetic analysis showed that the complete sequence clustered in a monophyletic group with the chimpanzee and gorilla sequences (Fig. 1A). Analysis of the C,

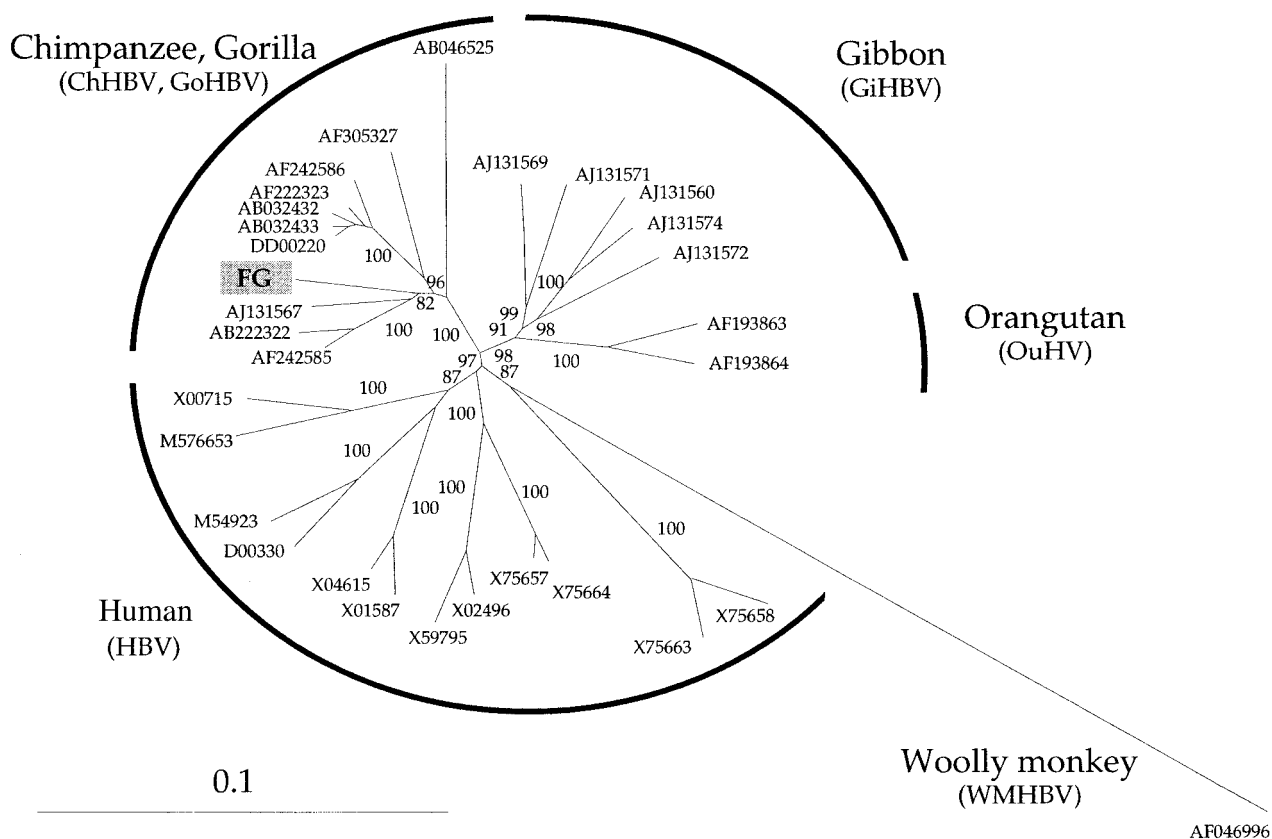
TABLE 1. Viral test results for chimpanzees

Virus(es)	Result for chimpanzee:			
	AK (kidney)	FG (liver)	RAS (lung)	SAD (lung)
SIVcpz	—	—	—	—
STLV-1	—	—	+	—
STLV-2	—	—	—	—
Simian foamy virus	—	+	+	+
Simian HBV	—	+	—	—
TTV circinovirus	+	+	+	+
Kaposi's sarcoma-associated herpesvirus	—	—	—	+
Adenovirus types 2 and 5	+	—	—	—
Adeno-associated virus	—	—	—	—
Simian virus 40	—	—	—	—
JC virus, BK virus	—	—	—	—
Parvovirus B19	—	—	—	—
Poxviruses	—	—	—	—
Papillomaviruses	—	—	—	—

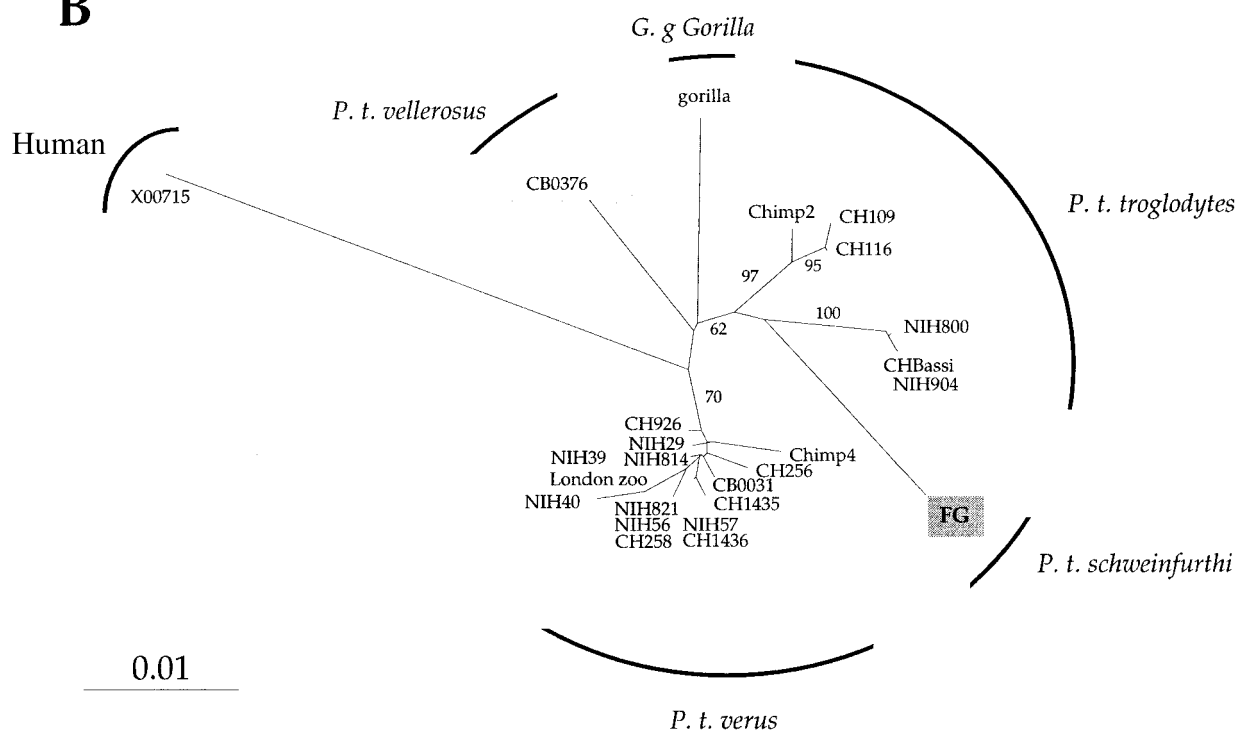
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† This paper is dedicated to the memory of Bill Hamilton.

A



# B



	10	20	30	40	50	60	70	80
<i>P. t. schweinfurthi</i> (FG)	MENITSGFLG	PLLVLQAGFF	LLTKILTIPO	SLDSWWTSLN	FLGGAPVCLG	QNSQSPTSNN	SPTSCPPICP	GYRWMCLRRF
<i>P. t. vellerosus</i>	..T.....	.....	.....	.....	.....	.....	.....	.....
<i>P. t. troglodytes</i>	.....	.....	.....	.....	.....	.....	.....	.....
<i>P. t. verus</i>	.....	.....	.....	.....	.....	.....	.....	.....
<i>G. g. gorilla</i>	..S.....	.....	.....	.....	.....	.....	.....	.....
	90	100	110	120	130	140	150	160
<i>P. t. schweinfurthi</i> (FG)	IIFLFILLLC	LIFLLVLLDY	QGMLPVCPLI	PGSSTTSTGP	CKTCTTPAHG	TSMFPSCCCT	KPSDGNCTCI	PIPSSWAFAR
<i>P. t. vellerosus</i>	.....	.....	.....	.....	.....Q..	..L.....	.....	.....K
<i>P. t. troglodytes</i>	.....	.....	.....	.....	.....Q..	..LI.....	.....	.....X..K
<i>P. t. verus</i>	.....	.....	.....	.....	.....Q..	..LI.....	.....	.....K
<i>G. g. gorilla</i>	.....	.....	.....	..T.....	.....T.Q..	..LI.....	.....	.....K
	170	180	190	200	210	220		
<i>P. t. schweinfurthi</i> (FG)	FLWEWASVRF	SWLSLLVPFV	QWFAGLSPTV	WLSVIWMMWY	WGPSLYNILS	PFLPLLPIFF	RLWVYI	
<i>P. t. vellerosus</i>	.....	.....A...	.....E....	.....	.....	..I.....	C.....	
<i>P. t. troglodytes</i>	.....	.....A...	.....	..L.....	.....	..I.....	C.....	
<i>P. t. verus</i>	.....	.....A...	.....	..LA.....	..N.....	..I.....	C.....	
<i>G. g. gorilla</i>	.....	.....A...	.....	.....	..N.....	..I.....	C.....	

FIG. 2. Alignment of the small S protein from chimpanzee HBVs with the sequence of the virus obtained from the animal FG (*P. t. schweinfurthi*). Only differences are shown. Grey boxes represent amino acid positions involved in determining the human HBV serodeterminants d/y, a, and r/w, respectively. The other sequences shown are from viruses isolated from *P. t. vellerosus* (sequence CB0376, accession number AF305327), *P. t. troglodytes* (sequence chimp2, accession number AF242585), *P. t. verus* from the London zoo (sequence DD00220), and *G. g. gorilla* (accession number AJ131567).

P, S, and X genes generated essentially the same tree, indicating that the virus was not a recombinant. As the database for the major viral surface antigen (HBsAg), encoded by the S gene, is the largest, a tree was constructed from 24 sequences (Fig. 1B). Even though the bootstrap values are not particularly strong, the sequence of the virus obtained from FG clustered more closely with the other viral sequences obtained from the *P. t. troglodytes* subspecies. This is perhaps not especially surprising, as *P. t. troglodytes* and *P. t. schweinfurthi* are the most closely related subspecies (4, 18).

The HBsAg protein sequence of the HBV obtained from chimpanzee FG was aligned with those of a number of chimpanzee and gorilla viruses (Fig. 2). It differed by 3 to 5%. For the human viruses, HBsAg is characterized by the a group-specific antigenic determinant and two sets of mutually exclusive subtype-specific sero-determinants, y/d and w/r (1, 12), resulting from two lysine/arginine (K/R) polymorphisms at positions 122 and 160 (15). Hence, there are four possible serotypes, adw, adr, ayw, and ayr, the last being extremely rare and confined to eastern Asia (3, 25). To date, the chimpanzee

HBsAg sequences have been uniform at these two sites, manifesting the equivalent of the adw subtype. The HBV sequence obtained from chimpanzee FG presents an arginine residue at position 160 typical of an adr subtype (Fig. 2). It is possible, therefore, that chimpanzee viruses might have the antigenicities of their human counterparts. Interestingly, orangutan and gibbon HBVs from Asia show the K/R polymorphism at position 160, although they have an invariant arginine at position 122, which is predictive of ayw and ayr serotypes (23). Hence, all four counterparts to the human polymorphisms may be found among great apes.

In terms of transmission of some of these viruses, it is perhaps worth noting that both FG and AK died after having shown influenza-like symptoms. This might bear on the finding of an adenovirus in AK. Among humans, HBV is highly infectious; presumably, therefore, FG would have been infectious also. The case of SAD is somewhat particular; he was killed after having attacked, bitten, and killed three small children. The STLV-1-positive animal, RAS, had been killed by a group of chimpanzees from a neighboring social community (24). His

FIG. 1. Phylogenetic analysis of HBV sequences. (A) Phylogenetic comparison of HBV sequences of viruses from chimpanzees, humans, and other nonhuman primates. Sequences were aligned by CLUSTAL W. Nucleotide sequence distances were determined with Dnadist of the Phylip package (version 3.5). Calculated distances were then used by applying the neighbor-joining method to pairwise sequence distances calculated by the Kimura two-parameter method to generate unrooted trees. Horizontal branch lengths are drawn to scale, with the bar indicating 0.1 nucleotide replacement per site. The final output was generated with Treeview. The number at each node represents the percentage of bootstrap replicates (out of 100). Only bootstrap values of  $\geq 80$  are given. The sequence of the virus obtained from FG was most closely related to those of chimpanzees and gorilla subtypes (0.5 to 0.7% nucleic acid divergence) and was distinct from the genotypes of human (10 to 15% divergence), gibbon ape (11% divergence), orangutan (11% divergence), and woolly monkey (28% divergence) viruses. (B) Phylogenetic comparison of HBV S-region sequences of viruses from chimpanzee subspecies. Horizontal branch lengths are drawn to scale, with the bar indicating 0.01 nucleotide replacement per site. The number at each node represents the percentage of bootstrap replicates (out of 100). Only bootstrap values of  $\geq 60$  are given. Sequences, identified by common names, and their GenBank accession numbers were as follows: CHBassi, AB046525; CB0376, AF305327; chimp4, AF242586; CH926, AF222322; CH256, AB032433; gorilla, AJ131567; CH109, AB222322; chimp2, AF242585; NIH800, AF222318; NIH904, AF222321; CH116, AF305328; NIH814, AF222319; NIH29, AF222312; NIH39, AF222313; NIH821, AF222320; NIH56, AF222316; NIH40, AF222314; CB0031, AF305326; NIH57, AF222317; CH1435, AF305329; CH1436, AF305330; CH258, AB032433; London zoo chimpanzee, DD00220; and the HBV subtype as an outgroup, X00715.

throat and testes had been torn out. Since monkey bites can transmit foamy viruses (8, 16), aggression by chimpanzees might also be conducive to the transmission of some of these viruses. However, as TTV, human T-cell leukemia virus type 1, HBV, and Kaposi's sarcoma-associated herpesvirus or human herpesvirus 8 are widely dispersed in human populations, it is not obvious that these animals represent a health hazard. Quite possibly, a pathologist is more vulnerable to infections than a zookeeper. Nevertheless, there is increasing contact between humans and chimpanzees via the bush meat trade (7). The number of viruses and, by extrapolation, the number of microbes (13) harbored by animals reemphasize the point that animals constitute pools of infectious agents. Given the highly related cellular biochemistries of chimpanzees and humans, the transfer of infections, perhaps in both directions, is probably facile.

In conclusion, screening for viruses is easy to perform and can extend our knowledge of the microbial floras among the great apes. The finding of an HBV in a *P. t. schweinfurthi* animal means that the virus is dispersed throughout the entire range of chimpanzee habitats from West Africa to the Great Lakes, unlike SIVcpz, which is found only among central African subspecies (2, 7, 17).

**Nucleotide sequence accession number.** The sequences of the complete HBV genome of animal FG can be found in GenBank under accession number AF498266.

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